44. A method of flow cytometry comprising contacting a population of cells with a compound of a claim 28 and detecting cells bearing receptors on their surfaces by detecting cells bound to said compound.

REMARKS

The applicants appreciate the Examiner's thorough examination of the application and request reexamination and reconsideration of the application in view of the preceding amendments and the following remarks.

The Examiner rejects claims 1, 3, and 10-27 under 35 U.S.C. §103(a) as being unpatentable over EPA 606 804 ('EPA '804) in view of Brunnett et al. However, because the applicants have canceled claims 1, 3, and 10-27, the Examiner's rejection of these claims is now moot.

The Examiner indicates that claim 5 would be allowable if rewritten in independent form. The applicants' have rewritten claim 5 and its base claim (claim 1) as new claim 28 to comply with the Examiner's indication of allowability. Accordingly, new claim 28 is allowable under 35 U.S.C. §103(a). Because claims 29 through 44 depend from claim 28, claims 29 through 44 are also allowable under 35 U.S.C. §103(a).

The Examiner rejects canceled claim 18 under 35 USC §112, ¶2 due to the use of the trademarks Bodipy™, Alexia™, and Iaedans™. The Examiner states that the use of the trademarks in claim 18 is improper since it continues to be descriptive of the product that is ultimately bound to the gastrin-releasing protein-based peptide. The Examiner requests the applicants cancel the trademarks from claim 18 and the specification and add the chemical name for the trademarks.

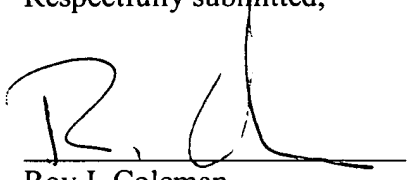
New claim 35 corresponds to originally filed claim 18 and has been amended, as shown above under AMENDMENT B, to reflect the chemical name of the compounds for BODIPY and IAEDANS. The applicants have also removed all reference to ALEXIA.

Specifically, "Bodipy" in the specification and new claim 45 have been replaced with 4,4-difluoro-4-bora-3a-diaza-s-indacene. IAEDANS in the specification and claim 35 has been replaced with 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid. Accordingly, claim 35 is definite and in accordance with 35 USC §112, ¶2.

Each of the Examiner's rejections has been addressed or traversed. Accordingly, it is respectfully submitted that the application is in condition for allowance. Early and favorable action is respectfully requested.

If for any reason this Response is found to be incomplete, or if at any time it appears that a telephone conference with counsel would help advance prosecution, please telephone the undersigned or his associates, collect in Waltham, Massachusetts, at (781) 890-5678.

Respectfully submitted,



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and Nle represents norleucine) (SEQ ID NO:5). The first residue is attached to the (C-X) moiety and is preferably chemically bound to the (C-X) moiety through the N-terminal amino acid. In still other preferred embodiments, the (C-X) bond is either C=O or C=S. In another preferred embodiment, the peptide may be amidated at the C-terminus. For example, the bombesin like peptides disclosed herein are amidated at the C-terminus.

In other preferred embodiments, the light-emitting moiety (R_1) is selected from the group including ~~Bodipy~~ 4,4-difluoro-4-bora-3a-diaza-s-indacene, fluorescein, fluoroscien isothiocyanate (FITC), Texas red, phycoerythrin, rhodamine, carboxytetramethylrhodamine, indopyras dyes, Cascade blue, coumarins, nitrobenzo-2-oxa-diazole (NBD), Lucifer Yellow, propidium iodide, CY3, CY5, CY9, dinitrophenol (DNP), lanthanide cryptates, lanthanide chelates, non-fluorescent dialdehydes (OPA, NDA, ADA, ATTOTAG reagents from Molecular Probes) which react with primary amines (N-term lys) in the presence of a nucleophile (i.e. CN^-) to form fluorescent isoindoles, ~~ALEXA dyes~~, dansyl dyes fluorescamine and dabcyl chloride, ~~IAEDANS~~ 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid, long lifetime dyes comprised of metal-ligand complexes (MLC) which consist of a metal center (Ru, Re, Os) and organic or inorganic ligands complexed to the metal such as such as $[Ru(bpy)_3]^{2+}$ and $[Ru(bpy)_2(dcbpy)]$, and the like and derivatives thereof. The light-emitting moiety can be attached to the peptide by reaction of a reactive side group (of the light-emitting moiety) with the N-terminal amino acid of bombesin-like peptide. Suitable reactive side groups include, by way of example only, indoacetamide, maleimide, isothiocyanate, succinimidyl ester, sulfonyl halide, aldehydes, glyoxal, hydrazine and derivatives thereof.

The above-identified compound is useful in the labeling of cell receptor sites, cell sorting, flow cytometry and performing fluoroimmunoassays. In another aspect, the invention provides a method for labeling a receptor having an affinity for a bombesin-like peptide by contacting the receptor with one or more of the compounds described above. Cell receptor sites, can be imaged by contacting candidate cell receptor sites with the

peptide moiety may be easily attached to the light-emitting moiety. Reactions for such modifications are described in, for example, *"Handbook of Fluorescent Probes and Research Chemicals - 5th Edition" (supra).*

The conditions used to modify amine moieties of the desired peptide will depend on the class of amine (e.g., aromatic, aliphatic) and its basicity. Aliphatic amines, such as the α-amino acid groups of lysine and arginine, are moderately basic and reactive with acylating reagents. The concentrations of the free base form of the aliphatic amines below pH 8 is very low; thus, the kinetics of acylation reaction of amines by isothiocyanates, succinimidyl esters, and other reagents is strongly pH-dependent. Although amine acylation reactions should usually be carried out above pH 8.5, the acylation reagents degrade in the presence of water, with the rate increasing as the pH increases. Therefore, a pH of 8.5-9.5 is usually optimal.

In general, thiols or alcohols react with alkylating groups (R'-Z) to yield relatively stable ethers or thiol ethers (R'-S-R), e.g., (C-X) is the α-carbon of R', with the leaving group Z preferably being a halogen (e.g., Cl, Br, I) and the like. In particular, the most common reagents for derivatization of thiols are haloacetyl derivatives. Reaction of these reagents with thiols proceeds rapidly at or below room temperature in the physiological pH range.

In general, any dye, porphyrin, fluorophore, or other light-emitting molecule may be complexed with the bombesin-like peptide. In preferred embodiments, the light-emitting moiety is selected from the group including ~~Bodipy~~ 4,4-difluoro-4-bora-3a-diaza-s-indacene, fluorescein, FITC, Texas red, phycoerythrin, rhodamine, carboxytetra-methylrhodamine, indopyras dyes, Cascade blue, coumarins, NBD, Lucifer Yellow, propidium iodide, CY3, CY5, and CY9, dinitrophenol (DNP), lanthanide cryptates, lanthanide chelates, non-fluorescent dialdehydes (OPA, NDA, ADA, ATTOTAG reagents from Molecular Probes) which react with primary amines (N-term lysine) in the presence of a nucleophile (i.e. CN^-) to

form fluorescent isoindoles, ALEXA dyes, dansyl dyes, fluorescamine and dabcyl chloride, IAEDANS, long lifetime dyes comprised of metal-ligand complexes (MLC) which consist of a metal center (Ru, Re, Os) and organic or inorganic ligands complexed to the metal such as such as $[Ru(bpy)_3]^{2+}$ and $[Ru(bpy)_2(dcbpy)]$, and the like and derivatives thereof. The synthesis and structures of several dyes which may be used are described in U.S. Patents 5,248,782; 5,274,113; and, 5,187,288, the contents of which are incorporated herein by reference. Other light-emitting moieties used in labeling or other applications may be attached to the peptide. For example, suitable light-emitting moieties are described in *"Handbook of Fluorescent Probes and Research Chemicals - 5th Edition"* by Richard P. Haugland 1994; and "Design and Application of Indicator Dyes", *Noninvasive Techniques in Cell Biology*: 1-20 by Richard P. Haugland et al., Wiley-Liss Inc. (1990), the contents of each of which is incorporated herein by reference.

In general, reactive groups on the light-emitting moiety, such as unsaturated alkyl groups or acylating moieties, will react with the modified peptide to form a dye/peptide bond. The chemical structure of the light-emitting moiety may affect the synthetic route used to synthesize the fluorescent bombesin-like peptide analog. It may be necessary, for example, to modify the light-emitting moiety so that it includes a reactive group prior to exposing this moiety to the desired peptide. Such side groups may include indoacetamide, maleimide, isothiocyanate, succinimidyl ester, sulfonyl halide, aldehyde, glyoxal and hydrazine derivatives. Amino acids including alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine may be labeled according to the method described herein.

The chemistry used to synthesize the fluorescent peptide is not greatly dependent upon the exact structure of the bombesin-like peptide analog. Thus, the general procedure outlined below may be used for most bombesin-like peptides. Attachment of